Studies on the Clinical Significance of Nonesterified and Total Cholesterol in Urine

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Summary. Gas-liquid chromatographic determinations of nonesterified and total urinary cholesterol were performed in 137 normals, 264 patients with various internal diseases without evidence of neoplasias or diseases of the kidney or urinary tract, 497 patients with malignancies and 236 patients with diseases of the kidney, urinary tract infections or prostatic adenoma with residual urine. A normal range (mean ± 2 SD) of 0.2–2.2 mg/24 hours nonesterified cholesterol (NEC) and of 0.3–3.0 mg/24 hours total cholesterol (TC) was calculated.

Values of urinary cholesterol excretion were independent of age and sex and did not correlate with cholesterol levels in plasma. Patients with various internal diseases, without evidence of neoplasias nor diseases of the kidney or obstruction of the urinary tract, showed normal urinary cholesterol excretions, as did patients with infections of the urinary tract.

However, elevated urinary cholesterol was found in patients with diseases of the kidney or urinary tract obstruction (prostatic adenoma with residual urine), malignant diseases of the urogenital tract and metastasing carcinoma of the breast. In patients with other malignant diseases urinary cholesterol was usually normal.

Lesions of the urothelial cell membranes are considered to be the most likely cause of urinary cholesterol hyperexcretion. The clinical value of urinary cholesterol determinations as a possible screening test for urogenital carcinomas in unselected populations is limited by lacking specificity, expensive methodology and low prevalence of the mentioned carcinomas, although elevated urinary cholesterol excretions have been observed in early clinical stages of urogenital cancers.

Key words: Urinary cholesterol – Diagnostic value – Diseases of the urogenital system

Untersuchungen zur klinischen Bedeutung des Freien und Gesamtcholesterins im Urin

Zusammenfassung. Gaschromatographische Bestimmungen von unverestertem und Gesamtcholesterin im Urin wurden bei 137 Normalpersonen, 264 Patienten mit verschiedenen internen Erkrankungen, ohne Hinweis für ein Malignom oder eine Erkrankung der Nieren und ableitenden Harnwege, 479 Patienten mit Malignomen und 236 Patienten mit Erkrankungen der Niere, Infektionen der ableitenden Harnwege oder Prostataadenom mit Restharn, durchgeführt.

Ein Normalbereich ($\bar{X}$ log ± 2 SD) von 0.2–2.2 mg/24 Stunden für unverestertes Cholesterin und von 0.3–3.0 mg/24 Stunden für Gesamtcholesterin wurde errechnet.

Die Ausscheidung des Cholesterins im Urin war unabhängig vom Alter und Geschlecht und korrelierte nicht mit den Cholesterinwerten im Plasma. Patienten mit verschiedenen internen Erkrankungen, ohne Hinweis für ein Malignom oder eine Erkrankung der Nieren und ableitenden Harnwege, zeigten eine normale Cholesterinausscheidung im Urin, wie auch Patienten mit Harnwegsinfektionen.

Dagegen wurden ein erhöhtes Urincholesterin bei Patienten mit Nierenerkrankungen oder Prostataadenomen mit Restharn, malignen Erkrankungen des Urogenitaltrakts, sowie bei metastasierenden Mammacarcinomen beobachtet.

Bei Patienten mit Malignomen anderer Lokalisation war die Cholesterinausscheidung im Urin gewöhnlich normal. Läsionen von Zellmembranen des Urothels können als die wahrscheinlichste Ursache einer gesteigerten Cholesterinausscheidung im Urin angesehen werden.

Der klinische Wert der Cholesterinbestimmung im Urin als möglicher Screeningtest für Urogenitalcarcinome in unselektierten Populationen ist begrenzt durch fehlende Spezifität, aufwendige Methodik und

insbesondere die niedrige Prävalenz der erwähnten Carcinome, obwohl erhöhte Cholesterinausscheidungen im Urin auch in Frühstadien von Urogenitalcarcinomen beobachtet werden konnten.

Schlüsselwörter: Cholesterin im Urin – diagnostischer Wert – Erkrankungen des Urogenitalsystems

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Anisotropic material is a well recognized component in the urine sediment of patients with the nephrotic syndrome (Bruger M, 1935; Zimmer JG et al., 1961, Klahr S et al., 1967). It has been pointed out that this microscopic material is apparently composed largely of cholesterol esters with a smaller proportion of free cholesterol (Zimmer JG et al., 1961). In addition to these findings there were some reports about increased urinary cholesterol in kidney diseases without nephrotic syndrome and various malignant diseases (Bloch E, Sobotka H, 1938; Sobotka H et al., 1939; Sobotka H et al., 1940; Trappe W, 1942; Bruger M, Ehrlich SB, 1943; Comings DE, 1963). Later studies demonstrated a lower incidence, about 10%, of hypercholesteroluria in cancer (Burchell MM et al., 1949; Burchell MM, Maclagan NF, 1949). Using a more specific mass fragmentometric method hyperexcretion of urinary cholesterol had been observed in single patients with prostatic or bladder carcinomas (Frick J, Spiteller G, 1968; Spiteller G et al., 1971). At the same time, a specific gas-liquid chromatographic method was developed for the determination of nonesterified urinary cholesterol (Vela BA, Acevedo HF, 1969). They determined elevated values of this steroid in patients with carcinomas of steroid hormone producing glands and their main target tissues (2, 3, 4, 5, 6). It was concluded that determinations of urinary cholesterol can be used as an additional biochemical test to detect these cancers.

Part of these results could be confirmed recently, demonstrating considerable diagnostic sensitivity of total urinary cholesterol determination in advanced prostatic cancer (Chu TM et al., 1975). However, as a result of gross deficiencies in patient selection, the clinical value of urinary cholesterol determinations is still not well defined.

Own previously published studies demonstrated considerable diagnostic sensitivity of nonesterified and especially total urinary cholesterol determination in prostatic cancer and papilloma or carcinoma of the bladder (Jüngst D et al., 1979a, b). Part of these results are included in the presented study which was carried out in an attempt to evaluate the clinical significance of nonesterified and total cholesterol determinations in urine with special regard to early stages of urogenital carcinomas.

In addition, urinary cholesterol measurements were performed in patients with nonmalignant diseases especially of kidney and urinary tract to investigate the diagnostic specificity of these parameters.

Materials and Methods

The study included a total of 1134 subjects. Normal range for nonesterified (NEC) and total urinary cholesterol (TC) was calculated using standard procedures and obtained from 137 clinically healthy, asymptomatic and biochemically normal individuals, 67 males and 70 females, covering an age range of 14 to 76 years.

The 264 patients with various internal diseases consisted of 75 with endocrine and metabolic, 68 with cardiovascular, 78 with gastrointestinal, 27 with respiratory and 16 with haemopoetic disorders. The presence of neoplasias, kidney diseases and prostatic adenoma with residual urine had been excluded in this second control group with reasonable certainty.

A total of 497 patients with a diagnosis of cancer, based on cytological or histological examinations, were included in the study. Only were urinary samples with macroscopically blood contamination discarded, since the presence of occult blood in urine did not alter the determination of urinary cholesterol.

Patients with carcinomas of the prostate, bladder and kidney had been classified according to the TNM-system and were divided into two groups ($T_1N_0M_0 - T_2N_0M_0$ and $T_1N_1M_0 - T_4N_4M_{1d}$). Patients with carcinoma of the cervix and corpus uteri were classified due to the FIGO, patients with breast cancer were divided into stage I–II and stage III–IV according to the ICRP. In patients with cancer of other origin staging had not been possible in all cases, therefore, a division in different groups, due to the extension of the disease, had not been done.

236 patients revealed diseases of the kidney, urinary tract infections or prostatic adenomas with residual urine which were diagnosed by standard clinical procedures.

Glomerular diseases of the kidney were present in 12, tubular diseases in 24 and vascular diseases in 11. 29 patients revealed an urolithiasis, 15 a renal cyst and 27 a nephrotic syndrome. Renal insufficiency of unknown etiology occurred in 29 patients, infections of the urinary tract in 32 and prostatic adenoma with residual urine in 57.

Nonesterified and total urinary cholesterol were analyzed in 2 ml aliquots of 24 h urine with a gas-liquid chromatographic assay using 4-androstendione as internal standard and peakheight ratio technique for quantitation. This method has been described in detail previously (Jüngst et al. 1979a).

Results

Normal Subjects

Based on the statistical analysis of the results, we obtained a mean excretion: 0.76 mg/24 h NEC and 0.92 mg/24 h TC in men and 0.84 mg/24 h NEC and 0.98 mg/24 h TC in women. The range for normal values was calculated as the mean ± 2 SD including 95% of the population. On the basis of these results, an upper normal limit: 2.2 mg/24 h NEC and 2.9 mg/24 h TC was determined in men and 2.3 mg/24 h NEC and 3.1 mg/24 h TC in women.
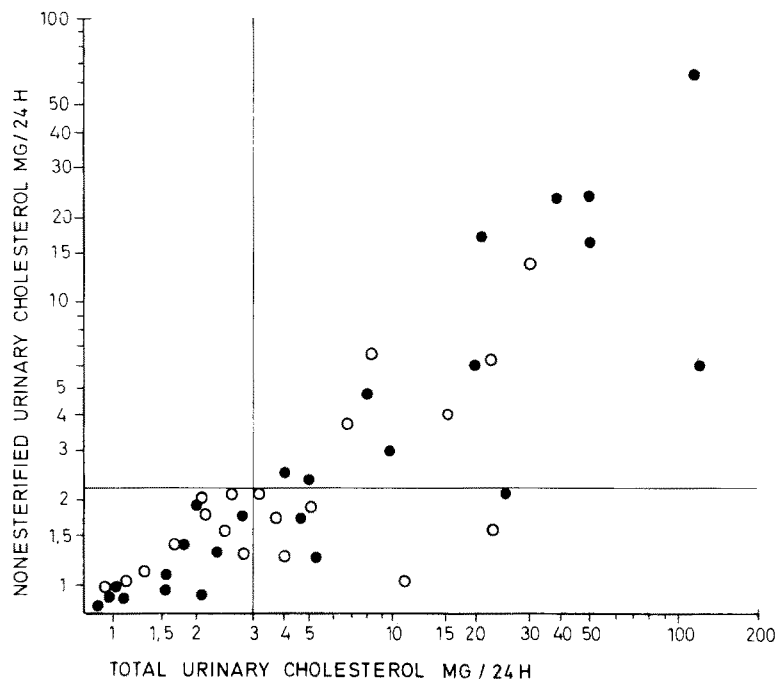
Fig. 1. Nonesterified and total urinary cholesterol in 45 patients with carcinoma of the kidney o stage $T_1N_0M_0 - T_2N_0M_0$, • stage $T_1N_1M_0 - T_4N_4M_{1d}$

Control Patients with Various Internal Diseases

In 264 patients with various internal diseases urinary excretion of NEC and TC were within the normal range, except 3 cases with slightly elevated levels of NEC and one patient with elevated TC (NEC range 0.2–2.4, median 0.7; TC range 0.3–3.1, median 1.0).

There was not a constant ratio between NEC and TC; in most cases nonesterified cholesterol was the major component of total urinary cholesterol. No correlation between urinary cholesterol excretion and cholesterol concentrations in plasma was obtained, even in patients with marked hypercholesterolaemia urinary cholesterol values were normal.

Carcinoma of the Prostate

In prostatic carcinoma $(T_1N_0M_0 - T_2N_0M_0)$ hyperexcretion of NEC was detected in 9 and of TC in 11 out of 24 patients (NEC range 0.4–24.2, median 1.2; TC range 0.5–51.4, median 2.2). In advanced clinical stages of the disease $(T_3N_0M_0 - T_4N_4M_{1d})$ elevated levels of NEC were measured in 25 and of TC in 27 out of 40 patients (NEC range 0.2–54.3; median 4.2; TC range 0.3–98.4, median 10.3).

Papilloma and Carcinoma of the Bladder

In papilloma and carcinoma of the bladder $(T_1N_0M_0 - T_2N_0M_0)$ hyperexcretion of NEC occurred in 23 and of TC in 29 out of 44 patients (NEC range 0.2–68.4, median 2.7; TC range 0.3–398.4, median 7.0).

In 21 patients with carcinoma of the bladder $(T_3N_0M_0 - T_4N_4M_{1d})$ elevated NEC was detected in 16 and elevated TC in 19 (NEC range 0.5–29.8, median 7.8; TC range 0.6–96.5, median 14.8).

Carcinoma of the Kidney

In 20 patients with carcinoma of the kidney $(T_1N_0M_0 - T_2N_0M_0)$ hyperexcretion of NEC occurred in 5 and of TC in 11 (NEC range 0.8–15.2, median 2.2; TC range 0.9–30.4, median 3.2). In advanced clinical stages of the disease $(T_1N_1M_0 - T_4N_4M_{1d})$ elevated values of NEC were detected in 11 and of TC in 14 of the 25 patients investigated (NEC range 0.7–64.3, median 1.8; TC range 0.7–124.4, median 4.4). Single values of these patients are illustrated in Fig. 1.

Carcinoma of the Breast

From 22 patients with cancer stage I–II, 5 showed slightly elevated values of NEC and 3 of TC, which could be observed in 21 and 19, respectively, of the 70 patients with clinical stage III–IV (NEC range 0.6–11.8, median 1.8; TC range 0.7–17.8, median 2.2). Single values are demonstrated in Fig. 2.

Carcinoma of the Uterus

From the 57 patients with carcinoma of the cervix 19 revealed elevated levels of NEC and 17 of TC (NEC range 0.6–19.6, median 1.8; TC range 0.7–35.4, median 2.2).
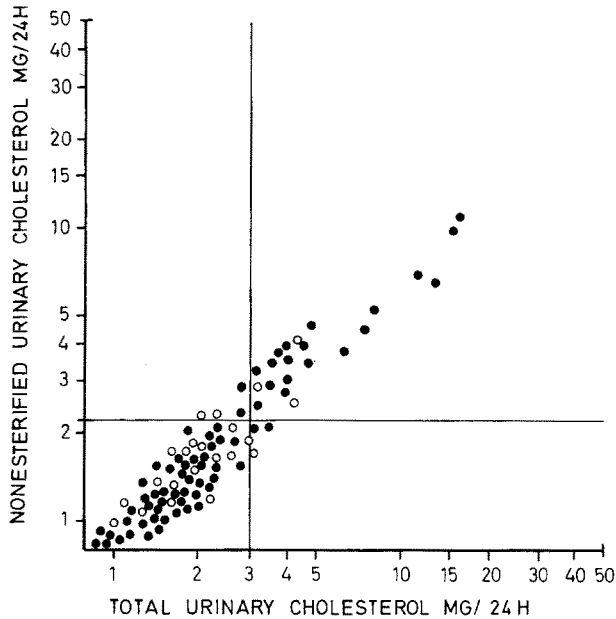
Fig. 2. Nonesterified and total urinary cholesterol in 91 patients with carcinoma of the breast. ○ stage I–II, ● stage III–IV
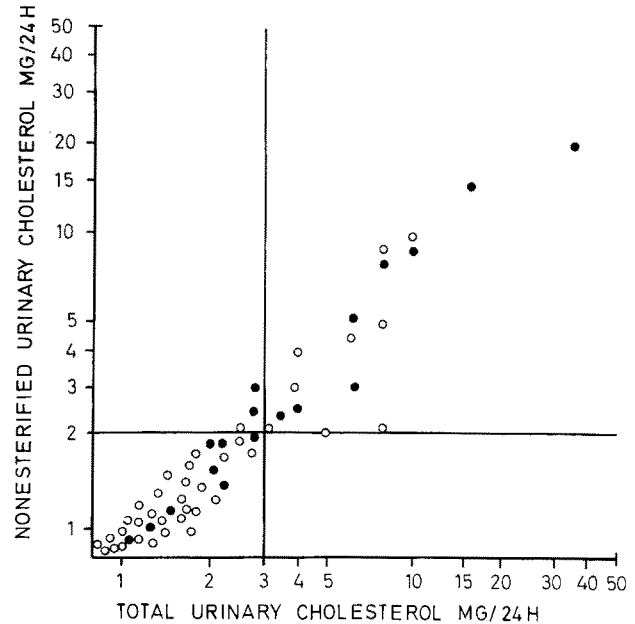

Fig. 3. Nonesterified and total urinary cholesterol in 57 patients with carcinoma of the cervix uteri. ○ stage I–II, ● stage III–IV

In 40 patients with carcinoma of the corpus uteri hyperexcretion of NEC occurred in 13 and of TC in 11 (NEC range 0.8–13.2, median 1.9; TC range 0.9–27.3, median 2.1). The individual results are illustrated in Fig. 3 and Fig. 4.

Carcinoma of the Ovary

6 out of 19 patients with carcinoma of the ovary revealed slightly elevated NEC or TC levels (NEC range 0.4–4.8, median 1.7; TC range 0.6–8.2, median 2.4).

Carcinoma of the Testes

In 21 patients with malignant diseases of the testes moderate hyperexcretion of NEC was observed in 5 and of TC in 6 patients (NEC range 0.4–3.8, median 1.8; TC range 0.5–12.4, median 2.3).

Other Malignant Diseases

The 16 patients with carcinoma of the bronchial tract showed normal urinary cholesterol values. 4 out of 28 patients with neoplasms of the gastrointestinal tract revealed slightly elevated levels of NEC and TC. In the 46 patients with malignant systemic diseases, hyperexcretion of NEC occurred in 7 and of TC in 9 cases (Fig. 5).

Diseases of the Kidney and Urinary Tract

In 47 patients with glomerular, tubular or vascular diseases of the kidney hyperexcretion of NEC took place in 12 and of TC in 20 (NEC range 0.5–6.6,
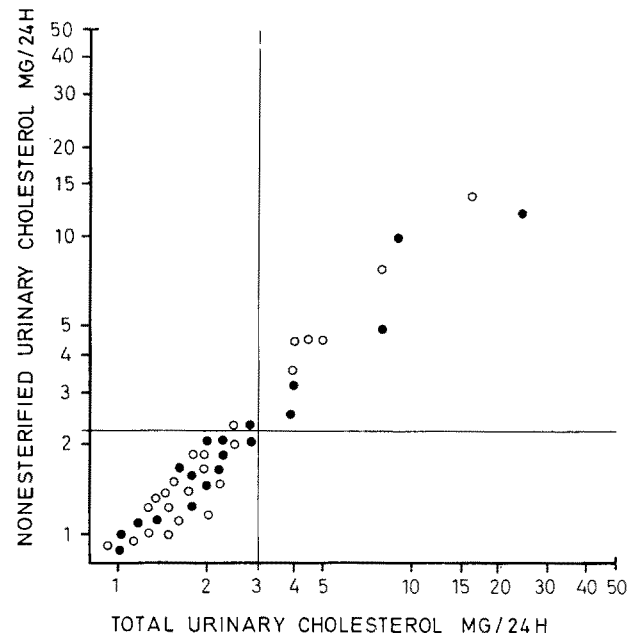

Fig. 4. Nonesterified and total urinary cholesterol in 40 patients with carcinoma of the corpus uteri. ○ stage I–II, ● stage III–IV

median 1.8; TC range 0.6–11.5, median 2.8) (Fig. 6).

From the 29 patients with urolithiasis 8 revealed elevated levels of NEC and 13 of TC, similar findings were obtained in 29 patients with renal insufficiency of unknown etiology (NEC range 0.3–9.8, median 1.5; TC range 0.4–31.5, median 2.6) (Fig. 7).

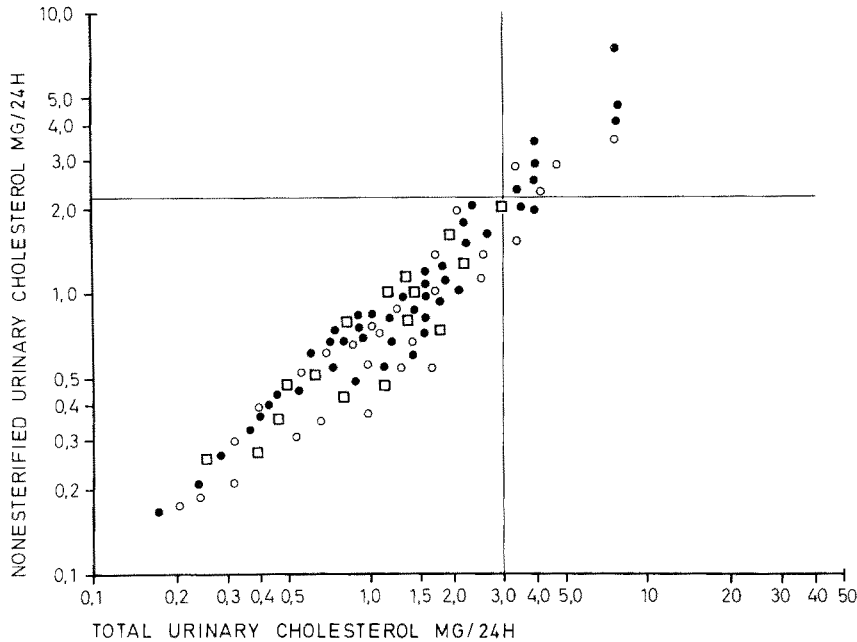In 27 patients with the nephrotic syndrome hyperexcretion of NEC was detected in 20 and of TC in

Fig. 5. Nonesterified and total urinary cholesterol in 16 patients with carcinoma of the bronchial tract □, 28 patients with carcinoma of the gastrointestinal tract o and 46 patients with malignant systemic diseases ●
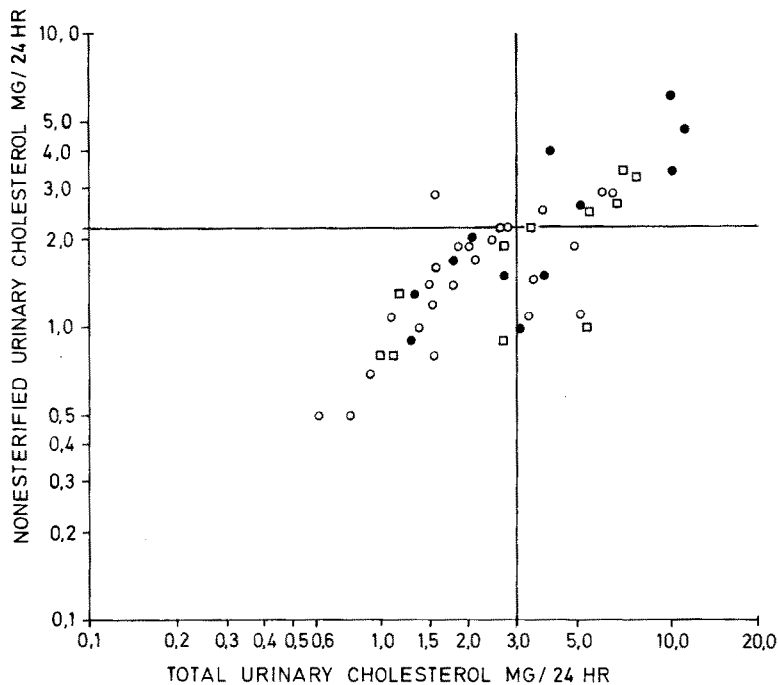

Fig. 6. Nonesterified and total urinary cholesterol in patients with glomerular ●, tubular o and vascular □ diseases of the kidney

25 (NEC range 0.8–38.2, median 4.4; TC range 1.0–93.5, median 12.6). Lower values of excretion were measured in 15 patients with renal cysts (NEC range 0.8–68.4, median 1.7; TC range 1.3–82.4, median 3.5) (Fig. 8).

32 patients with common infections of the urinary tract revealed normal urinary cholesterol excretion in all cases.

Benign Prostatic Hyperplasia with Residual Urine

The results obtained in the study of patients with prostatic hyperplasia and residual urine have been published in detail before, hyperexcretion of NEC was observed in 24 and of TC in 37 out of 57 patients (NEC range 0.3–49.0, median 2.0; TC range 0.4–112.3, median 5.8).

Discussion

Our results demonstrated pathologic values in prostatic carcinoma $T_1N_0M_0 - T_2N_0M_0$ in 37% of NEC and 45% of TC. In advanced clinical stages percentage of elevated values increased to 62.5% for NEC and 67.5% for TC.
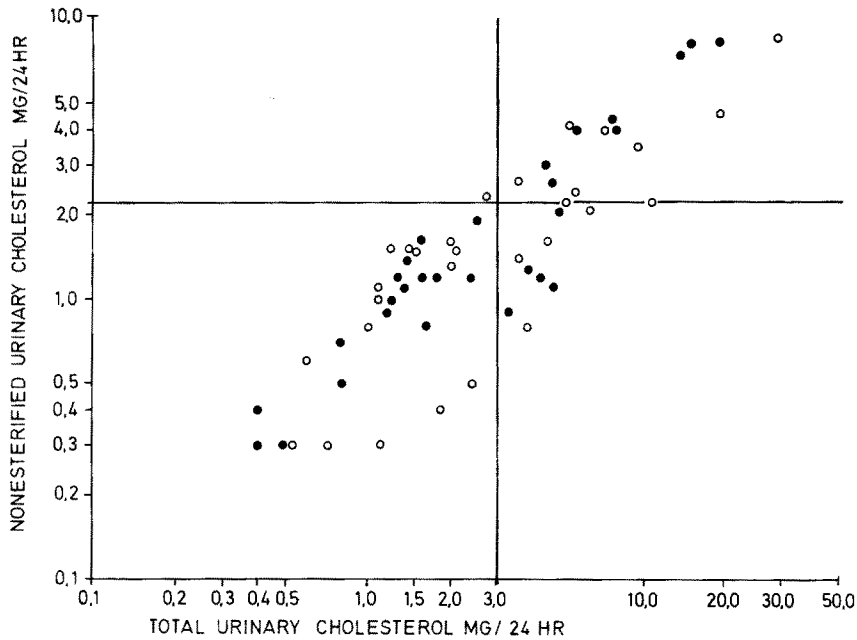
Fig. 7. Nonesterified and total urinary cholesterol in patients with urolithiasis ● and renal insufficiency of unknown etiology ○
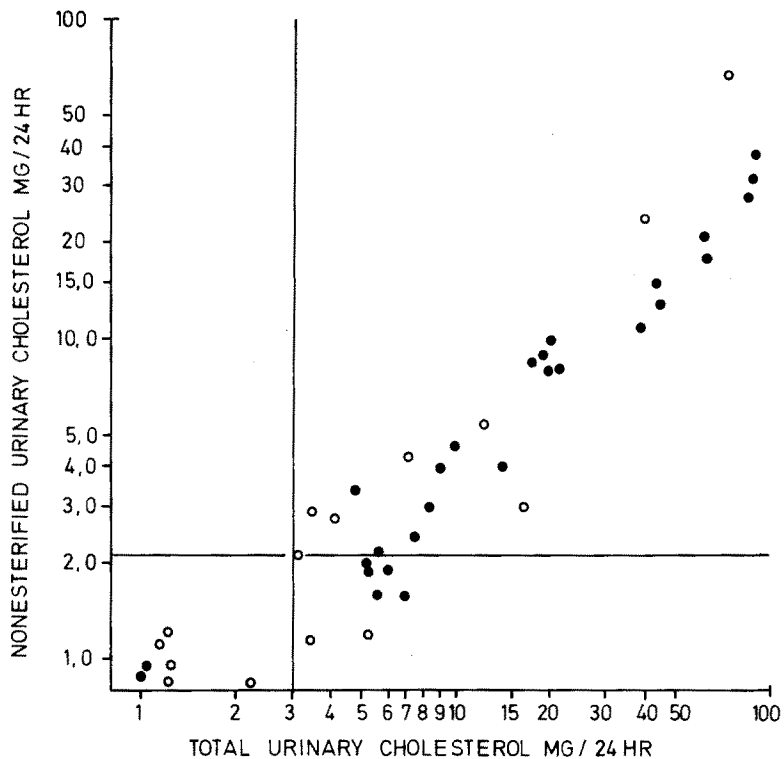

Fig. 8. Nonesterified and total urinary cholesterol in patients with nephrotic syndrome ● and renal cysts ○

These findings are in good agreement with those reported recently (Belis and Cenedella 1979). In papilloma of the bladder NEC hyperexcretion was present in 56%, elevated levels of TC were determined in 68%, similar to data obtained in early stages of carcinoma of the bladder. In advanced clinical stages hyperexcretion of NEC was observed in 76% and of TC in 90%. In early clinical stages of carcinoma of the kidney hyperexcretion of NEC was present in

about 25% and of TC in about 55%. Percentage of elevated values increased to 44 of NEC and 56 of TC in further advanced stages. In patients with carcinoma of the cervix or corpus uteri elevated levels of NEC or TC were detected in approximately 30%, comparable to the results of previous studies (Acevedo et al. 1971; Acevedo et al. 1975). In early clinical stages of patients with breast cancer, marked hyperexcretion of urinary cholesterol has not been detected

in contrast to patients with metastases. Malignant diseases of testes and ovary revealed moderate hyperexcretion of urinary cholesterol in 24–31%, in carcinomas of other origin slightly elevated urinary cholesterol has been observed in about 12%.

In view of these results it is concluded that the determination of especially total cholesterol in urine has a considerable diagnostic sensitivity for carcinomas of the urogenital system. It is more pronounced in patients with cancer of the kidney, prostate and bladder than uterus, ovary and testis.

However, urinary cholesterol hyperexcretion could not be considered to be a specific sign of urogenital malignancies. Findings also demonstrate elevated values in patients with prostatic adenoma and residual urine. In addition, hyperexcretion of urinary cholesterol is a common finding in kidney diseases, irrespective of etiology or any other clinical features. However, it is important to note that common infections of the urinary tract are not associated with hyperexcretion of urinary cholesterol.

Discussing the clinical value of the method presented one can state that increments of urinary cholesterol are not a specific sign of urogenital malignancies but are usually caused by malignant or nonmalignant diseases of the urogenital system.

The application of this method as a possible screening test in cancer detection programs seemed to be limited only little by lacking specificity or expensive methodology. In our opinion most important is the fact of low prevalence of urogenital carcinomas in unselected populations which would lead to low predictive values for a positive urinary cholesterol test and the presence of urogenital malignancies. This does not necessarily exclude the possibility that the urinary cholesterol method could be successfully applied in preselected populations with increased prevalence of urogenital carcinomas.

Although the physiologic mechanisms of urinary cholesterol excretion are not known, an excretion as a protein bound complex was suggested on the basis of ultrafiltration studies (Acevedo et al. 1970).

Enhanced production of lipoproteins by the malignant cells had been discussed to cause hyperexcretion of urinary cholesterol in cancer of steroid hormone producing glands or their main target tissues (Acevedo et al. 1973). In addition the presence of immuncomplex nephritis, a well known disease in cancer patients, could be responsible partially for elevated urinary cholesterol values (Kerkhoven et al. 1973; Sutherland and Mardiney 1973; Eagen and Lewis 1977).

However, in our opinion it is most likely that alterations of the urothelial cell membranes with subsequent loss of lipoproteins are responsible for hyperexcretion of urinary cholesterol irrespective of the underlying disease. Only in patients with the nephrotic syndrome seems the positive correlation between urinary protein and urinary cholesterol to support the suggestions that enhanced filtration of plasma lipoproteins, especially the HDL-fraction, is responsible for increased lipiduria (Klahr et al. 1967; de Mendoza et al. 1976; Bing and Starup 1935).

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